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Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae-Ceropegieae)

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Based on morphological, karyological and DNA sequence data the circumscription of *Lavrania* Plowes, *Larryleachia* Plowes and *Notechidnopsis* Lavranos and Bleck is reconsidered. The data presented point to an intermediate position of *Notechidnopsis* between the more distantly related *Larryleachia* and *Lavrania*. Keeping the three genera distinct is the best reflection of the complex relationships within this group.

Introduction

In the over 30 genera of stem-succulent stapeliads (Ceropegieae) presently recognised (Albers and Meve, in press a), the generic treatment of the highly succulent 'smooth-stemmed' *Trichocaulon* species was often subject to controversial systematic considerations (Plowes 1996, Bruyns 1993, 1999a).

NE Brown (1878) introduced the genus *Trichocaulon* NE Br. to accommodate two small-flowered (spiny) species of *Hoodia* Sweet ex Decne. He subsequently added some more species, including smooth-stemmed representatives (Brown 1909). Nel (1933) added the third element under discussion here, *Trichocaulon columnare* Nel. This concept was adopted by AC White and B Sloane (1937). Later, *Trichocaulon columnare*, together with *Caralluma tessellata* Pillans, were transferred to the new genus *Notechidnopsis* by Lavranos and Bleck (1985) to separate these two rhizomataceous species lacking the smooth stems of *Trichocaulon* sect. *Cactoidea* White and Sloane as well as the spines of *Hoodia*. In 1986, Plowes described *Lavrania haagnerae* Plowes, the fourth ceroid multi-ribbed taxon known in the southwestern corner of southern Africa.

Plowes (1992a), in a first attempt to split the undisputedly heterogeneous *Trichocaulon sensu* NE Brown and White and Sloane, transferred all *Trichocaulon* taxa with spines to *Hoodia*. This generic transfer has been adopted by Bruyns (1993), although the number of accepted species differs considerably between the two treatments. The remaining smooth-stemmed *Trichocaulon* taxa needed a new name, because the type species of *Trichocaulon*, the spiny *T. piliferum* L..f., had also been transferred to *Hoodia*. Plowes (1992a), therefore, accommodated them in the new genus *Leachia* Plowes. However, this name is illegitimate because it is a later homonym of *Leachia* Cassini (Asteraceae). In the process of correcting his nomenclatural mistake, Plowes (1992b) repeated it by publishing *Leachiella* Plowes as substitute name for *Leachia* Plowes. Again, this generic name is illegitimate, since *Leachiella* Kugrens is an older name for a red alga. This unhappy situation was resolved by Bruyns (1993) with the transfer of the smooth-stemmed *Trichocaulon* taxa to the hitherto monotypic *Lavrania* Plowes. However, to reflect the phylogenetic distance of the two species groups, Bruyns (1993) created two different sections, *Lavrania* sect. *Lavrania*, for *L. haagnerae*, and sect. *Cactoidea* (AC White and B Sloane) Bruyns for the smooth-stemmed former *Trichocaulon* species. However, *Trichocaulon* sect. *Cactoidea* is a 'nomen nudum', so that this name is not valid either. In 1996, Plowes made a third attempt by proposing the genus name *Larryleachia* Plowes for *Lavrania* sect. *Cactoidea* (illeg. *Leachia* and illeg. *Leachiella*). But even with this now validly published generic name, taxonomic stability in this group was still not achieved. The transfer of *Lavrania* (incl. *Larryleachia*) to *Hoodia* by Halda (1998) caused temporary regression. Unfortunately Halda neither considered *Notechidnopsis* nor offered any new arguments why these species should be added to *Hoodia*; which in its present circumscription is a well-defined and independent genus.

Bruyns (1999a) probably was not aware of Halda's nomenclaturally correct transfers when he again 'clarified' the status of *Larryleachia* transferring *Larryleachia* to *Lavrania* and placing all species of *Larryleachia*, including its type *Stapelia cactiformis* Hook., in his newly described section *Lavrania* sect. *Cactoidea* Bruyns. The new section, again, is based on the type *S. cactiformis*.

The focus of this study is on the complex relationships between *Lavrania*, *Larryleachia sensu* Plowes and to the sympatric, cereoid and non-spiny, bitypic *Notechidnopsis*. Plowes (1986) discussed *Notechidnopsis* as putative closest

relative of *Lavrania s.str.*, while Bruyns (1999b) regards *Notechidnopsis* as a monophyletic genus most closely related to *Pectinaria* Haw. The following options are examined:

- Should *Notechidnopsis*, *Lavrania s.str.* and *Larryleachia* be regarded as separate genera as proposed by Plowes (1996)?
- Should *Lavrania s.str.* and *Larryleachia* (*Lavrania* sect. *Cactoidea*, respectively) be united to *Lavrania s.l.* while *Notechidnopsis* is kept separate (*sensu* Bruyns 1993, 1999a)?
- Should *Notechidnopsis* be united with *Lavrania* and/or *Larryleachia*?

Materials and Methods

Materials

The material used in this study, including voucher specimens, is summarised in Table 1.

Chromosome counts

Chromosome numbers were established from adventitious root tip squash preparations. The root tips were pretreated in 0.002M hydroxyquinoline for 4hrs at 20°C (Tjio and Levan 1950), fixed in Carnoy's solution for 24hrs at 20°C and stained with carmine for 24hrs at 60°C (Snow 1963).

DNA extraction and PCR

DNA was isolated from fresh stem tip tissue according to Doyle and Doyle (1987). PCR primers and protocol for the plastid *trn*T-*trn*L and *trn*L-*trn*F spacers correspond to Taberlet *et al.* (1991).

The entire Internal Transcribed Spacer region (ITS) of ribosomal DNA was amplified using the flanking primers ITS4 and ITS5 following a slightly modified protocol from Baldwin (1992). The 25µl reactions contained (in the order of addition): 15.8µl sterile water, 2µl $MgCl_2$ (25mM), 1.5µl dNTP3 (20mM), 2.5µl 10x buffer, 1µl ITS4 (1µM), 1µl ITS5 (1µM), 0.2µl Taq DNA polymerase (1 Unit, Qiagen), 1µl template DNA. PCR was conducted with an initial 3' at 94°C preliminary denaturing, and 30 reaction cycles (1' at 94°C, 1' at 57°C, 3' at 72°C).

Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. All sequences have been deposited at EMBL Nucleotide Sequence Database (Accession Numbers AJ402116–AJ402162).

Data analysis

Sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was cleaned manually. The 'Taberlet' alignment (available from the authors) comprises 12 taxa and 1711 characters [814 sequence characters in the *trn*T-*trn*L intron (primers a–b), 512 sequence characters between the two *trn*L-exons (primers c–d), and 384 sequence characters and 1 indel in the *trn*L-*trn*F intron (primers e–f)]; 26 data cells are unknown. The ITS alignment comprises 11 taxa and 655 data cells. Both alignments are either available from the authors or can be viewed on the World Wide Web (http://www.uni-bayreuth.de/departments/planta2/).

Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0d65 (PPC), Swofford (1998) on a MacIntosh Powerbook G3. For parsimony analysis, gaps were coded as 'missing' and excluded by deletion of 'missing/ambiguous' and 'uninformative'. In a second analysis, indels 3bp and longer were coded separately as 'present/absent'. Bootstrap search (1 000 replicates) was conducted under the 'full heuristic' search option, starting trees were obtained by 'stepwise addition' and 'random' addition sequence with 10 replicates; swapping algorithm was set to 'TBR' and 'MulTrees'. Jackknife resampling (1 000 replicates) was set to 50% deletion, and 'Jac' resampling; the other settings were identical to the bootstrap settings.

Distances were computed using the Neighbor-joining algorithm. All sequence characters were analysed using the three options under 'DNA-RNA distances': 'uncorrected (p)', 'Jukes-Cantor' and 'Kimura-2-parameter'.

As the results of analyses of the ITS and the 'Taberlet' dataset were largely contradictory, a partition homogeneity test (as included in PAUP version 4.0b3a) was conducted showing that the datasets are highly significantly discordant ($P = 0.01$). Assigning triple weight to the 'Taberlet' data to compensate for the lesser number of parsimony informative characters of this dataset lifts reduces the discordance ($P = 0.06$); still, a combination of the datasets does not seem advisable.

Table 1: Voucher and locality information for plant material used in this study

Species	Origin	Voucher	
Caralluma edulis (Edg.) Benth	Oman: Dhofar	*Butler* C312	UBT
Ceropegia nilotica Kotschy	Kenya: Mbolo Hill	*Masinde* 836	MSUN
Gymnema sylvestre (Retz.) Schult. (Marsdenieae)	Cameroon: E Mokolo	*Meve* 919	B, UBT
Lavrania haagnerae Plowes	Namibia: Khowarib Gorge	*Haagner* sub *Plowes* 5046	PRE, MSUN
Larryleachia cactiformis (Hook.) Plowes	South Africa: Numees	*Teissier* 097	UBT
Larryleachia perlata (Dinter) Plowes	South Africa: Richtersveld	*Jürgens* s.n	UBT
Notechidnopsis columnaris (Nel) Lavranos and Bleck	South Africa: Hellskloof	*Albers and Meve* 30	MSUN
Notechidnopsis tessellata (Pillans) Lavranos and Bleck	South Africa: Nieuwoudtville	*Meve* 256	MSUN
Pectinaria articulata (Ait.) Haw. ssp. *borealis* Bruyns	South Africa: Hellskloof	*Albers and Meve* 32	UBT
Piaranthus barrydalensis Meve	South Africa: Muiskraal	*Meve* et al. 128	K, MSUN
Piaranthus comptus NE Br.	South Africa: Klaarstroom	*Albers* sub K 1123	MSUN
Stapelia glanduliflora Masson	South Africa: S Klawer	*Albers and Meve* 04	MSUN

Results

Character assessment

Morphological characters

In his recent paper, Bruyns (1999a) accurately discussed the diagnostic value of eleven morphological characters stressed by Plowes (1996) to argue for the separation of *Larryleachia* from *Lavrania*. Bruyns (1999a) concludes that the only useful differences between both groups lie in the inflorescence position (basal in *Lavrania* versus apical/subapical in *Larryleachia*), and the arrangement of the stem tubercles (in regular rows in *Lavrania* versus irregular in *Larryleachia* — where tubercle arrangement is in fact spiral, at least in young plants). In the same paper, Bruyns shows figures of *Lavrania haagnerae* stems with subapical inflorescences, so that only the regularly arranged stem tubercles remain significant. In *Notechidnopsis* inflorescence position is subapical and the tubercles are linearly arranged. However, comparison and discussion of additional characters is indispensable (Table 2). Most conspicuous are the extremely sunken stomata in the rather smooth epidermis of *Lavrania* (Figure 1A), while stomata are not sunken, though slightly overtopped by the bulging surrounding epidermis in *Notechidnopsis* (Figure 1B) and *Larryleachia* (cf. Bruyns 1993: Figure 5G).

The number of rows (ribs) overlap, (6–10 in *Notechidnopsis*, 10–12 in *Lavrania*, and 12–20 in *Larryleachia*) rendering this character unreliable for taxonomic conclusions. Shape of sepals, flower buds, corolla (Figures 2A–C) and translators, as well as the basic epidermal pattern of the adaxial corolla lobe face are similar in all three groups and, therefore, not of taxonomic value.

Chromosomes

All three genera under consideration were investigated karyologically. All species possess 2n = 22 chromosomes, reflecting the situation in *ca.* 90% of stapeliad taxa (Albers and Meve 1991). However, chromosome size varies considerably. *Larryleachia* chromosomes are rather small, measuring only 0.97µm in *L. marlothii* (N.E. Br.) Plowes (voucher: *Albers and Meve* 45 [MSUN]; figure 3A) and 0.86µm in *L. picta* (N.E. Br.) Plowes (voucher: *Albers and Meve* 78, [MSUN, UBT]). *Lavrania haagnerae* has the largest chromosomes with an average length of 1.40µm (voucher: *Haagner* s.n. sub *Plowes* 5046, clonotype material [PRE, MSUN]; figure 3C). *Notechidnopsis* (1.13µm) is in-between (voucher: *N. tessellata*, *Meve 255* [MSUN, UBT]; Figure 3B).

DNA sequence analysis

Taberlet

Exclusion of 'uninformative' and 'missing/ambiguous' characters results in 13 parsimony-informative sequence characters.

Exhaustive search results in a single tree (1 = 17, CI = 0.8235, HI = 0.1765, RI = 0.8846, RC = 0.7285; Figure 4A). Neither inclusion of the single gap character nor inclusion of the two 'missing/ambiguous' characters changes the topology of the tree. Both *Notechidnopsis* species are sister to *Larryleachia*. There is no character difference between the two *Notechidnopsis* species nor between the two *Larryleachia* species. A single transition separates *Notechidnopsis* from *Larryleachia*, while *Lavrania haagnerae* differs from *Notechidnopsis* by a single transition and two transitions from *Larryleachia*.

ITS

Gymnema (and other Marsdenieae) are too distantly related to the remainder of the genera to allow an analysable alignment of ITS sequences. Instead, *Ceropegia nilotica* and *Caralluma edulis* were chosen as outgroups following the results of analysis of the 'Taberlet' dataset. Exclusion of 'uninformative' and 'missing/ambiguous' characters results in 39 parsimony-informative characters. Exhaustive search results in a single most parsimonious tree (1 = 53, CI = 0.8113, HI = 0.1887, RI = 0.8214, RC = 0.6664; Figure 5A). Here, *Larryleachta* is sister to *Piaranthus*; the two *Notechidnopsis* species are widely separated. The two *Larryleachia* species differ by a single transversion, while they differ from *L. haagnerae* in 17 resp. 18 positions, the same number of differences as between the *Larryleachia* species and *Piaranthus*. The two *Notechidnopsis* species

Table 2: Morphological characters and character states

Character	*Lavrania*	*Notechidnopsis*	*Larryleachia*
Growth	sympodial	sympodial	monopodial
Rhizomes	not present	present	not present
Number of rows of tubercles	10–12	6–10	12–20
Tubercles	flat	conical	conical
Leaf rudiments (cf. Bruyns 1993: Figure 5; Figure 1A, this paper)	short and acute, not sunken in groove, without stomata	short and acute (or nearly absent), not sunken in groove, without stomata	thickish and distinctly sunken in an adaxial groove of tubercle, with stomata
Stomata (of stem epidermis) (Figure 1B)	sunken	not sunken	not sunken
Corona tissue	firm, nectariferous	firm, nectariferous	membranous, not nectariferous
Interstaminal corona (Ci) (cf. Bruyns 1993; Figures 2A–C, this paper)	more or less entire to slightly bidentate	more or less entire to slightly bidentate	bifid into subulate lobules
Follicles (cf. Bruyns 1993, own obs.)	small, *ca.* 3–4mm diam.	intermediate, *ca.* 5–8mm diam.	stout, *ca.* 8mm diam.
Hypocotyl (cf. Bruyns 1993, 1999b)	without groove	with slight groove	with distinct groove

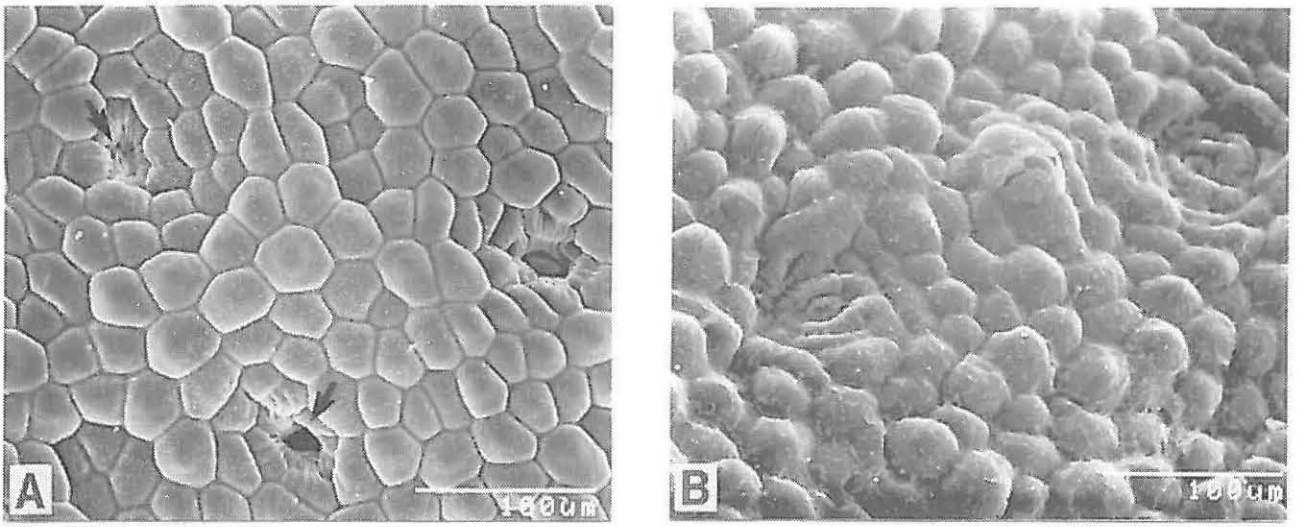

Figure 1: Stem epidermis surfaces. A. *Lavrania haagnerae*, arrows: sunken stomata; B. *Notechidnopsis tessellata* (SEM. A from Plowes 5046, B from Meve 255)

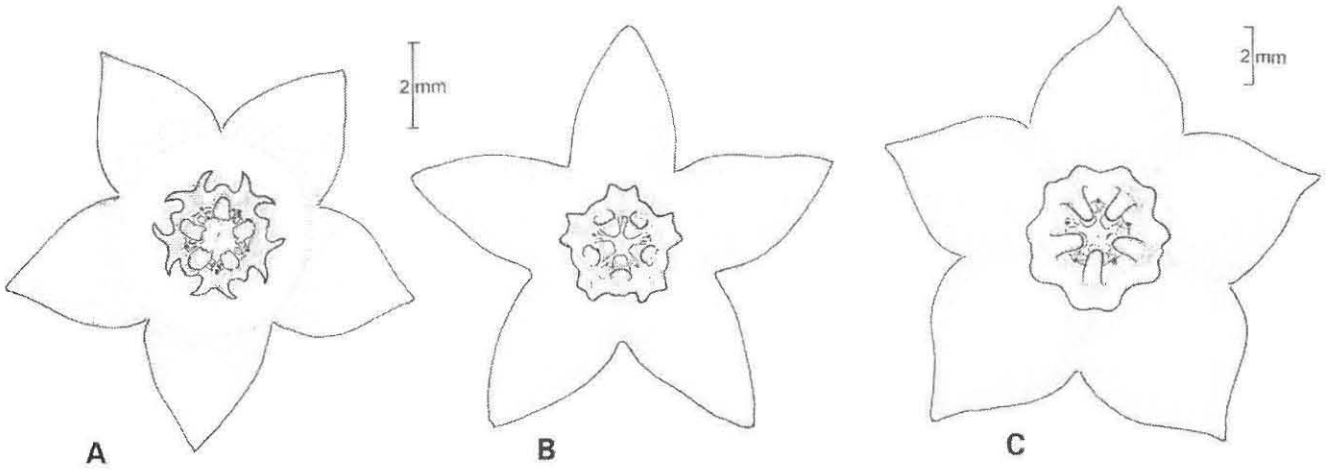

Figure 2: Flowers in top view (drawn without hairs and papillae of corolla surface). A. *Larryleachia perlata* (*Albers* sub K 1361 [MSUN]; B. *Notechidnopsis tessellata* (*Meve* 255 [MSUN]); C. *Lavrania haagnerae* (*Plowes* 5046 [PRE, MSUN]). Drawn by U Meve

differ in 11 positions from each other, *L. haagnerae* differs in 6 positions from *N. tesselata*, 13 from *N. columnaris*.

The distance analyses (neighbour-joining) confirm the topology of the parsimony analyses for the main clades for both 'Taberlet' and ITS data sets. Independent from the algorithm selected, sequence characters produce identical distance trees (Figures 4B, 5B).

Discussion

The taxonomic value of the 'morphological differences' between the three taxa has been intensively discussed in Plowes (1996) and Bruyns (1999a). Apart from the truly sunken stomata in *Lavrania*, a unique feature in the whole Ceropegieae (Meve, unpubl.) and a character not discussed by the two authors, the lack of stomata on the rudimentary leaves in *Lavrania* and *Notechidnopsis* in contrast to their presence in *Larryleachia* should not be neglected.

Chromosomes in the Asclepiadoideae are small and comparitively uniform, they vary from about 0.7 to 1.7µm on the average. Ceropegieae chromosomes are *ca.* 1.0µm long (Albers and Meve, in press b). Variation in the average length is limited within single stapeliad genera and varies closely around 1.1µm in well-circumscribed genera such as *Echidnopsis* (unpubl. data). Only in heterogeneous stapeliad genera such as *Caralluma* or very large genera such as *Huernia* considerable size deviations occur (Albers and Meve, in press b; Meve, unpubl. data). A generic fusion of

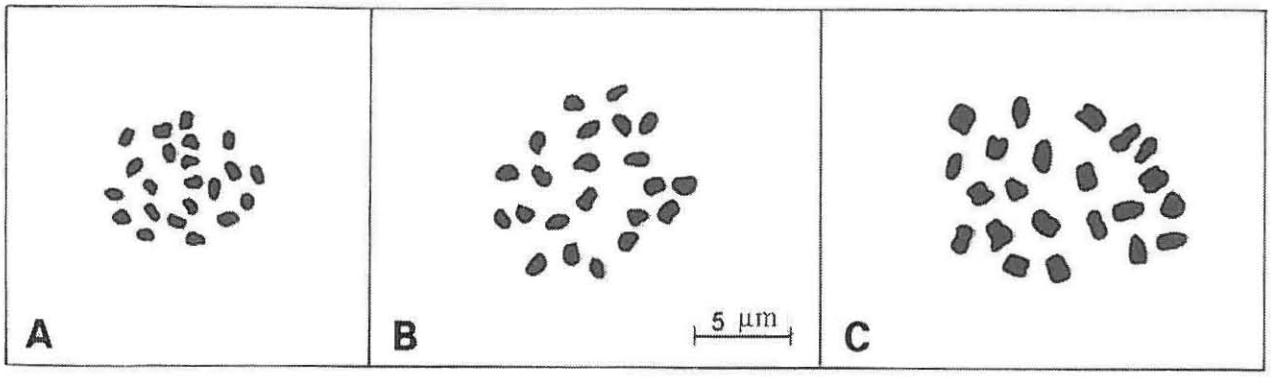

Figure 3: Mitotic metaphase plates showing 2n = 22. A. *Larryleachia marlothii* (*Albers and Meve* 45); B. *Notechidnopsis tessellata* (*Meve* 255); C. *Lavrania haagnerae* (*Plowes* 5046)

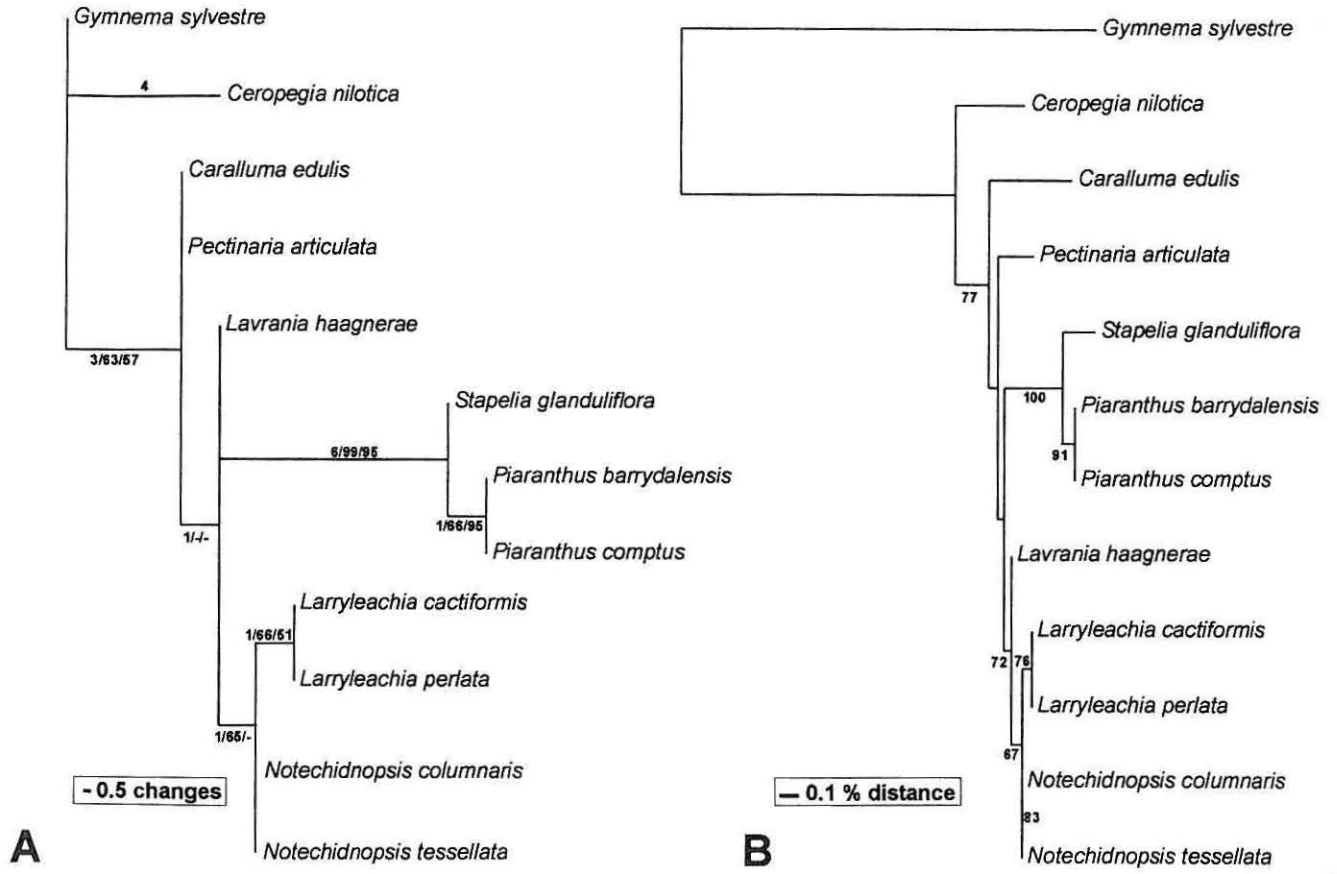

Figure 4: A. The single most parsimonious tree (I = 17, CI = 0.8235, HI = 0.1765, RI = 0.8846, RC = 0.7285) resulting from exhaustive analysis of 'Taberlet' sequence data. Numbers indicate branch length/ bootstrap percentage (1 000 replicates)/ jackknife values (1 000 replicates). B. Neighbour-joining tree (Kimura-2-parameter); numbers indicate bootstrap percentages (1 000 replicates)

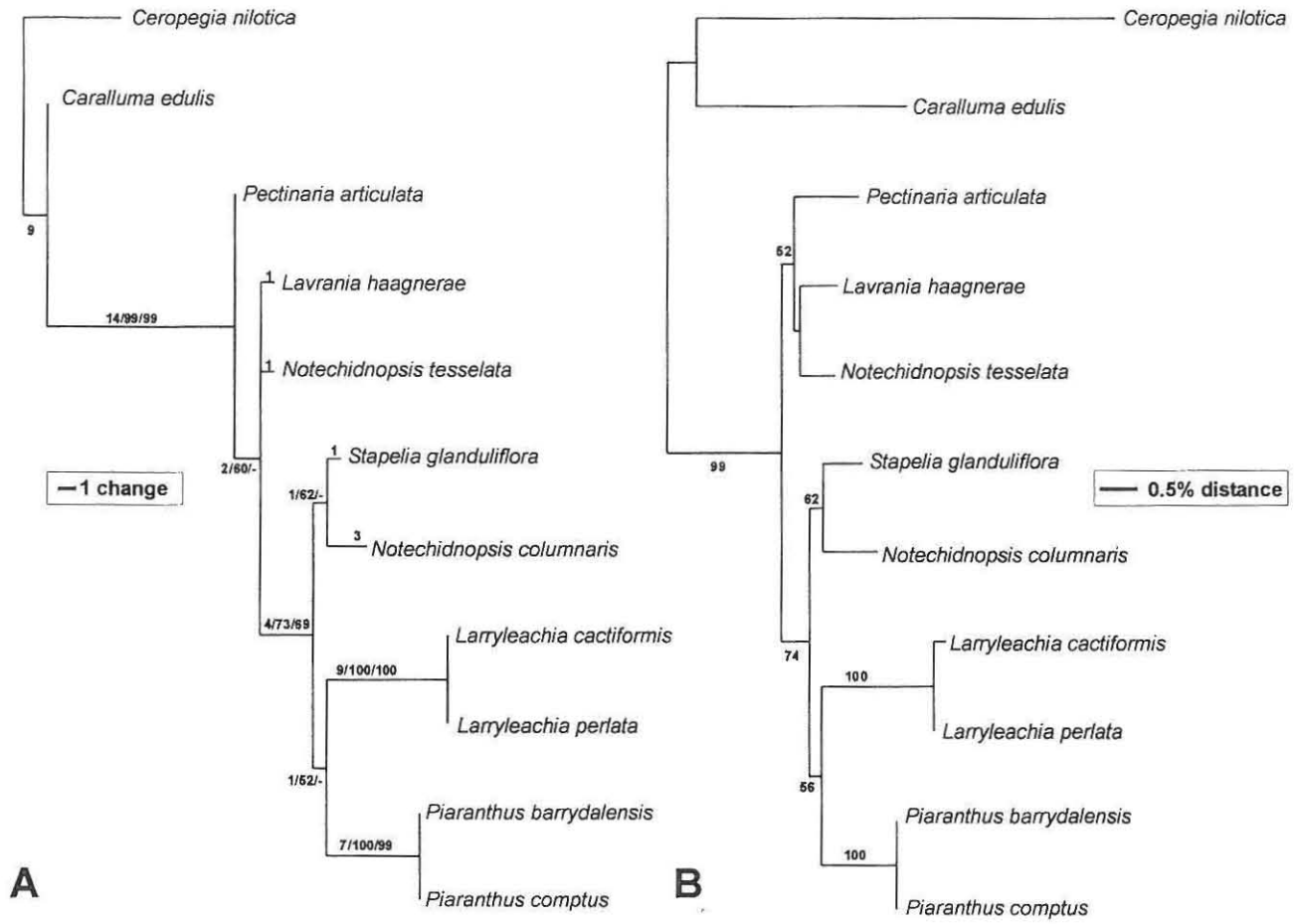

Figure 5: A. The single most parsimonious tree (1 = 53, CI = 0.8113, HI = 0.1887, RI = 0.8214, RC = 0.6664) resulting from exhaustive analysis of *ITS* sequence data. Numbers indicate branch length/bootstrap percentage (1 000 replicates)/ jackknife values (1 000 replicates) B. Neighbour-joining tree (Kimura-2-parameter); numbers indicate bootstrap percentages (1 000 replicates)

Larryleachia characterised by small-sized chromosomes (on average 0.91µm) with *Lavrania* (*L. haagnerae*: chromosomes 1.40µm large) is problematic from the karyological point of view. Average chromosome size in *Notechidnopsis* (*N. tessellata*: 1.13µm) is intermediate.

The homogeneity in each of the molecular datasets, the internal transcribed spacer of nuclear ribosomal DNA (ITS) and the plastid *trn*T-*trn*L and *trn*L-*trn*F spacers ('Taberlets'), point to a very close relationship of all taxa investigated. As a consequence of the incongruency between these two data sets an indisputable phylogeny of the three taxa cannot be generated. The 'Taberlet' data revealed no variation between the two *Notechidnopsis* taxa, and put both species, which do not form a clade, in a sister-group position to the *Larryleachia* clade; *Piaranthus* is sister to *Stapelia glanduliflora* and *Lavrania haagnerae* is sister to both the *S. glanduliflora/Piaranthus* clade and the *Notechidnopsis /Larryleachia* clade. In the ITS dataset the position of most genera is changed with *N. columnaris* sister to *Stapelia glanduliflora* and the *N. columnaris/S. glanduliflora* clade sister to the *Larryleachial/Piaranthus* clade. *L. haagnerae* and *N. tessellata* again, are sister to all of the above. How can these results be interpreted?

Regarding vegetative characters, namely size, shape of seedlings, stem tubercles and leaves, the two species of *Notechidnopsis* are not as uniform as those of the much larger genus *Larryleachia*. Bruyns (1999b), nevertheless, regards *Notechidnopsis* as a monophyletic genus. Regarding floral morphology, however, there is less heterogeneity and both *Notechidnopsis* species are artificially crossable (Meve, unpubl. data). Explaining the vegetative and molecular incongruencies, hybridogenous effects (cytoplasmic introgression) are possibly of importance. This idea is supported by distributional data, since all three elements under discussion share one common core distribution area in the semi-arid to arid regions of western Namibia and northwestern South Africa.

Morphological matrocliny is widespread and easily observed in stapeliads. If artificial cross-pollination leads to bastard plants, these typically exhibit more character states of the mother plant than of the pollen donor. Maternal heredity of the plastids (the father's contribution to the zygote is

limited to the bare nucleus genome; no paternal plastids are introduced into the zygote) is most likely responsible for this effect and might well be the reason for the contradictory evidence of the two sequence data sets. Such problems of incongruency in DNA sequence data sets has been the topic of some recent studies (Roelofs and Bachmann 1997, Mayer and Soltis 1999), which have favoured effects of introgression as the most likely explanation for such discrepancies.

Taxonomy

Morphological, karyological and sequence data point to an intermediate position of *Notechidnopsis* between *Larryleachia* and *Lavrania*. *Lavrania* including *Larryleachia* in the sense of Bruyns (1999a) would be paraphyletic without including at least *Notechidnopsis*, as the 'Taberlet' data suggest, or a much wider range of taxa, as the ITS data suggest, a rather unhappy situation from the taxonomical point of view. Neither molecular nor karyological evidence favours an inclusion of *Larryleachia* in *Lavrania*, neither as section nor as subgenus. Morphological arguments might be found for a generic fusion of *Notechidnopsis* with *Larryleachia* (common character states: conical tubercles, slightly bulging stem epidermis with stomata not sunken), or of *Notechidnopsis* with *Lavrania* (common character states: sympodial growth, acute leaf rudiments without stomata, nectariferous corona, non-subulate interstaminal corona lobes, smooth hypocotyl). From the morphological point of view, a fusion of *Lavrania* with *Notechidnopsis* is clearly better supported, but 'Taberlet' sequence data support a fusion of *Notechidnopsis* with *Larryleachia*. However, as ITS data clearly contradict both options, effects of putative lineage sorting in *Lavrania* and/or *Notechidnopsis* must be taken into consideration. Until their nature is better understood, the best reflection of phylogeny is to keep the three genera distinct:

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Type: *Lavrania haagnerae* Plowes: 123 (1986).

Larryleachia Plowes, Excelsa 17: 5 (1996).
Leachia Plowes, Asklepios 56: ii (1992), *nom. illeg.* [*non Leachia* Cassini 1822 (Asteraceae)]. *Leachiella* Plowes, Asklepios 57: 15(1992), *nom. illeg.* [*non Leachiella* Kugrens 1892].
Trichocaulon sect. *Cactoidea* A.C. White and B. Sloane, Stapelieae 3: 991 (1937), *nom. nud. Lavrania* sect. *Cactoidea* (A.C. White and B. Sloane) Bruyns, Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 115: 245 (1993), *nom. invalid. Hoodia* sect. *Cactoidea* (A.C. White and B. Sloane) Halda, Acta Musei Richnoviensis Sect. natur. 5: 31 (1998), *nom. invalid.* Basionym: *Trichocaulon* sect. *Cactoidea* A.C. White and B. Sloane.
Lavrania sect. *Cactoidea* Bruyns, South African Journal of Botany 65: 305 (1999).
Type: *Larryleachia cactiformis* (Hook.) Plowes: 5 (1996).
Basionym: *Stapelia cactiformis* Hook.

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Type: *Notechidnopsis tessellata* (Pillans) Lavranos and Bleck: 255 (1985). Basionym: *Caralluma tessellata* Pillans.

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Bruyns PV (1993) A revision of *Hoodia* and *Lavrania* (Asclepiadaceae-Stapelieae). Botanische Jahrbuecher fur Systematik Pflanzengeschichte und Pflanzengeographie **115**: 145–270

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Bruyns PV (1999b) A systematic analysis with notes on the taxonomy of *Notechidnopsis* (Apocynaceae: Asclepiadoideae). Kew Bulletin **52**: 327–345

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